

REMARKS

The specification has been amended herein to add reference to sequence identifiers introduced in response to the Notice to File Missing Parts of Nonprovisional Application mailed on January 17, 2001. A marked-up copy of the pages affected reflects the additions in red ink. The amendments to the application add no new matter. A paper copy and CRF of the replacement sequence listing is enclosed.

In connection with the Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the content of the attached paper copy and the enclosed computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same; and
2. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter.

Respectfully submitted,

Date: June 7, 2001

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Enclosure

subcloned into the pT7Blue TA vector (Novagen). A Hind III-Not I fragment containing the human mGluR2 cDNA was then subcloned into the Bluescript SKII(-) plasmid (Stratagene). This construct is referred to as phmGluR2.

5 C. phGα_q

A full length human Gα_q cDNA was amplified from human cerebral cortex Quick-Clone cDNA (Clontech) using PCR primers based on the human Gα_q cDNA sequence (Genbank Accession # 4504044). The obtained PCR fragment was subcloned into the Bluescript SKII(-) plasmid (Stratagene). This construct is referred to as phGα_q.

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D. phmGluR8

The cDNA encoding the full length human mGluR8 cDNA (Stormann *et al.*, U.S. Patent Nos. 6,051,688, 6,077,675, and 6,084,084) is harbored in the Bluescript SKII(-) plasmid (Stratagene). This construct is referred to as phmGluR8.

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E. ph8SPmGluR4

A full length human mGluR4 cDNA was amplified from human cerebellum MarathonReady cDNA (Clontech) using PCR primers based on the human mGluR4 cDNA sequence (Genbank Accession #X80818). The obtained PCR fragment was cloned into the pT7Blue TA vector (Novagen). A 2977 bp BamHI fragment containing the human mGluR4 cDNA was then subcloned into the vector pcDNA3.1/Hygro+ (Invitrogen). This construct is referred to as phmGluR4.

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Next, the predicted signal peptide of mGluR4 was replaced with the predicted signal peptide and 87 bp of 5' UTR from phmGluR8 using a recombinant PCR strategy similar to those described above. The first reaction used a phmGluR8 construct with two primers, 3.1-535F (sense 21-mer, complementary to vector sequence upstream of the hmGluR8 insert; sequence 5'-ggcattatgccaggtacatga-3'), and the hybrid primer 8/4RP (antisense 42-mer, containing 21 nucleotides complementary to human mGluR8 and 21 nucleotides complementary human mGluR4; sequence 5'-caagcctctcttccaggcattttctccacaggtgtattgc-3'). These primers were used to amplify a 469 bp PCR fragment of human mGluR8.

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In a separate PCR reaction using phmGluR4 as template, a 472 bp fragment of

human mGluR4 was amplified using a hybrid primer 4/8RP (sense 42-mer, exactly complementary to primer 8/4RP) and oligo mG4-472R, (antisense 18-mer, complementary to the human mGluR4 cDNA; sequence 5'-ctgaagcaccgatgacac-3'). The two PCR products generated from the above two reactions were annealed together in equimolar ratios in the presence of the external primers mG4-472R and 3.1-535F, and Turbo Pfu DNA polymerase (Stratagene).

The resulting chimeric PCR product was digested with NarI and NheI (New England Biolabs) and subcloned into phmGluR4 digested with the same two restriction enzymes. The sequence of the resultant chimeric construct, ph8SPmGluR4, was verified by ABI automated DNA sequence analysis.

The replacement of the predicted signal peptide of mGluR4 with that of mGluR8 greatly increased the activity of this receptor in *in vitro* assays

II. G α q β 5

The cDNA encoding the human G α q β 5 cDNA (Conklin et al (1993) Nature 363:274-77) is harbored in the Bluescript SKII(-) plasmid (Stratagene). This construct is referred to as G α q β 5. The nucleic acid and amino acid sequences for G α q β 5 are provided by SEQ. ID. NOs. 28 and 29 respectively.

III. phCaR/hmGluR2

This chimera contains the extracellular domain of the human CaR and transmembrane domain and intracellular cytoplasmic tail of human mGluR2. The chimeric junction between the CaR and hmGluR2 was created using a recombinant PCR strategy similar to those described above.

The first reaction used two primers, CA1156 (sense 19-mer, corresponding to nucleotides 1156-1174 of human CaR), and the hybrid primer CA/2 (antisense 42-mer, containing 21 nucleotides complementary to nucleotides 1774-1794 of human CaR and 21 nucleotides complementary to nucleotides 1660-1680 of the human mGluR2). These primers were used to amplify a 659 bp PCR fragment of human CaR.

In a separate PCR reaction using phmGluR2 as template, a 692 bp fragment of the human mGluR2 was amplified using a hybrid primer 2/CA (sense 42-mer, exactly complementary to primer CA/2) and oligo 2-2330m, (antisense 23-mer, complementary to

mer, containing 18 nucleotides complementary to nucleotides 2806-2823 of phGABA_BR2 and 18 nucleotides complementary to nucleotides 1-18 of hGα_qo5). These two complementary areas flank a 9 nucleotide sequence coding for 3 alanine sequences with a unique NotI restriction site. These primers were used to amplify a 200 base-pair PCR
5 fragment.

In a separate PCR reaction, part of hGα_qo5 was amplified using a hybrid primer R2/Gα_qo5(+) (sense 45-mer), exactly complementary to R2/Go5(-) and XbaI-Go5 primer (22-mer containing 22 nucleotides complementary to nucleotides 873-895 of hGα_qo5). These primers were used to amplify a 914 base-pair PCR product. The two PCR products
10 generated from the above two reactions were annealed together in equimolar ratios in the presence of the external primers; XcmI-R2 and XbaI-Go5, and Pfu polymerase (Stratagene).

The resulting chimeric PCR product was digested with the restriction endonucleases XcmI and XbaI (New England Biolabs) and subcloned into phGABA_BR2
15 digested with the same two restriction enzymes. The resulting clone was then digested with HindIII and XbaI and subcloned into phGα_qo5 cut with HindIII and XbaI resulting in the chimeric hGABA_BR*AAA*Gα_qo5. The chimeric junction between the C-terminus hGABA_BR1a, the Ala linker, and the N-terminus of hGα_qo5 was created using a recombinant PCR strategy similar to those described above.

20 To construct hGABA_BR1a*AAA*Gqo5, the first reaction used a commercially available T7 primer (Novagen) and the NtI hGBR1 primer (CAGAGTCATGGCGGCCGCTTATAAAGCAAATGCACTCG)/corresponding to nucleotide numbers 1-9 of hGα_qo5 and nucleotide numbers 2863-2883 of hGABA_BR1a.

25 C. phmGluR8//CaR*AAA*Gα_qi5

A linker encoding three alanine residues was incorporated into the phmGluR8//CaR*Gα_qi5 construct by mutagenesis (Stratagene QuickChange Mutagenesis Kit), exactly as described in Section A, above to create phmGluR2//CaR*AAA*Gα_qi5. The same primers, 2CQ+LP and 2CQ+LM, were used for this mutagenesis. Restriction
30 enzyme analysis and DNA sequence analysis confirmed the insertion of the 9-nucleotide linker (GCGGCCGCC) between the C-terminus of phmGluR8//CaR and the N-terminus of Gα_qi5. This construct was designated phmGluR8//CaR*AAA*Gα_qi5.

D. ph8SPmGluR4//CaR*AAA*G α _{i5}

This chimera contains the extracellular and transmembrane domains of the human 8SPmGluR4 construct and intracellular cytoplasmic tail of human CaR fused to G α _{i5} through the three alanine residue linker.

The chimeric junction between the human 8SPmGluR4 and hCaR was created using a recombinant PCR strategy similar to those previously described. The first reaction used two primers, mG4-2028R (sense 19-mer, corresponding to nucleotides of human 8SPmGluR4; sequence 5'-catctaccgcatcttcgag-3'), and the hybrid primer 4CT (antisense 42-mer, containing 21 nucleotides complementary to human 8SPmGluR4 and 21 nucleotides complementary human CaR; sequence 5'-acgcacctcctcgatggtgttctgctccgggtggaagaggat-3'). These primers were used to amplify a 549 bp PCR fragment from human 8SPmGluR4.

In a separate PCR reaction, using phmGluR2//CaR*AAA*G α _{i5} as a template, a 743 bp fragment of the human CaR*AAA*G α _{i5} was amplified using the hybrid primer CT4 (sense 42-mer, exactly complementary to primer 4CT) and oligo Gaqi58R, (antisense 21-mer, complementary to G α _{i5} cDNA; sequence 5'-ctcgatctcgtcgttgatccg-3'). The two PCR products generated from the above two reactions were annealed together in equimolar ratios in the presence of the external primers mG4-2028R and Gaqi58R, and Pfu DNA polymerase (Stratagene).

The resulting chimeric PCR product was digested sequentially with KpnI and NotI (New England Biolabs) and subcloned into ph8SPmGluR4 prepared with the same two restriction enzymes. This intermediate construct was known as ph8SPmGluR4//CaR(no stop). In the final cloning step, a fragment containing the G α _{i5} cDNA was released from phmGluR8//CaR*AAA*G α _{i5} using the restriction enzymes ApaI and NotI (both New England Biolabs) and subcloned into the ph8SPmGluR4//CaR(no stop) construct, which was prepared with the same restriction enzymes. The sequence of the resultant chimeric construct, ph8SPmGluR4//CaR*AAA*G α _{i5}, was verified by ABI automated DNA sequence analysis.

VIII. phmGluR8//CaR Construct

This chimera contains the extracellular and transmembrane domains of human mGluR8 linked to the intracellular cytoplasmic tail domain of the human CaR. The